

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problems Mailbox.**

STIC-ILL

386,987 NO

From: Canella, Karen  
Sent: Tuesday, March 05, 2002 6:31 PM  
T: STIC-ILL  
Subject: ill order 09/819,193

Art Unit 1642 Location 8E12(mail)

Telephone Number 308-8362

Application Number 09/819,193

1. Cancer Research:  
1981 May, 41(5):1978-1983  
1988 Apr 1, 48(7):1864-1873
2. Anticancer Research:  
1987 Jul-Aug, 7(4B):781-789  
1997, Vol. 17, No. 3C, pp. 1973-1983
3. Gynecologic Oncology:  
1990 May, 37(2):188-199  
1992 Jun, 45(3):273-278  
1991, Vol. 42, No. 1, pp. 39-43  
1982, Vol. 13, No. 2, pp. 50-57
4. Pathobiology:  
1997 Jul-Aug, 65(4):177-183  
1993, 61(2):67-76  
1992, 60(1):33-41
5. Jikeikai Med J:  
1989, 36(4):303-316  
1994, 41(4):407-415
6. Sapporo Med J:  
1988, 57(6):603-612  
1996, 65(5):433-444
7. Acta Obstetrica et Gynecologica Scandinavica, 1995, 74(5):330-335
8. Journal of Medicine, 1997, 28(3-4):175-190
9. Experimental and Molecular Pathology, 2000 Dec, 69(3):175-191
10. International Journal of Cancer, 15 Mar 2001, 91(6):778-782
11. Experimental Cell Research, 1992 Aug, 201(2):262-272

Thanks!

Jikeikai Med J 1994; 41: 407-15

## AROMATASE ACTIVITY OF HUMAN GYNECOLOGICAL CARCINOMA CELL LINES

Akio TADA\*, Junji NAKAMURA\*\*, and Hiroshi SASAKI\*

*\*Department of Obstetrics and Gynecology,  
The Jikei University School of Medicine and*

*\*\*Research Institute of Life Science Snow Brand Milk Products Co.*

(Received for publication, August 8, 1994)

### ABSTRACT

Human cancer cell lines derived from endometrium (HEC-59, HHUA, Ishikawa, OMC-2), uterine cervix (CAC-1, OMC-4), ovary (2780, 2008, HRA), and breast (MCF-7) were examined for aromatase activity and the effect of estradiol and testosterone on DNA synthesis. Aromatase activity was high (more than 500 fmol/10<sup>7</sup> cells/24 hrs) in the cell lines OMC-2 and MCF-7, moderate (100-499 fmol/10<sup>7</sup> cells/24 hrs) in OMC-4, HRA, HEC-59, Ishikawa, and CAC-1, and low (less than 100 fmol/10<sup>7</sup> cells/24 hrs) in HHUA, A2780, and 2008. A significant stimulation of DNA synthesis (250% increase of [<sup>3</sup>H]-thymidine uptake) by estradiol (10<sup>-8</sup> M) was observed in HEC-59, OMC-2, MCF-7, Ishikawa, and HRA. Stimulation of DNA synthesis by estradiol was moderate (115-249%) in OMC-4. Estradiol-dependent increase of DNA synthesis was not observed in HHUA, CAC-1, 2008, and A2780. The cell lines which showed the estradiol dependency in DNA synthesis, namely HEC-59, OMC-2, MCF-7, Ishikawa, and HRA, also presented testosterone dependency. These data suggest that some gynecological carcinomas may possess an aromatase-dependent growth stimulation system.

(Jikeikai Med J 1994; 41: 407-15)

Key words: gynecological neoplasms, cell lines, aromatase activity, estrogen dependency, testosterone dependency

多田 聖郎, 中村 純二, 佐々木 寛

Mailing address: Akio TADA, Department of Obstetrics and Gynecology, The Jikei University School of Medicine, 3-25-8, Nishi-Shinbashi, Minato-ku, Tokyo 105, Japan.

### INTRODUCTION

The incidence of endometrial carcinoma has recently been increasing<sup>1)</sup>. Obesity, which frequently correlates with a high serum estrogen level<sup>2,3)</sup>, is a known risk factor for endometrial carcinoma. Many endometrial carcinomas, especially well-differentiated adenocarcinomas, are estrogen-dependent with estrogen receptors. And they frequently respond well to hormonal therapy with progesterone receptors<sup>4,5)</sup>.

Human breast carcinoma, which is also an estrogen dependent tumor as well as endometrial carcinoma<sup>6,7)</sup>, has been reported to have aromatase activity<sup>8,9)</sup>. Aromatase is an enzyme which converts androgen to estrogen. And aromatase exists in a number of human tissues, including placenta, ovary<sup>10)</sup>, breast<sup>11,12)</sup>, adipose tissue<sup>13,14)</sup>, brain<sup>15,16)</sup>, and uterine endometrium<sup>17)</sup>. Many researchers have reported a positive link between aromatase activity and estrogen-dependent carcinogenesis. The glandular epithelium of the uterine endometrium, where endometrial carcinoma originates, contains both aromatase<sup>18)</sup> and estrogen receptors. It is a reasonable assumption that endometrial carcinomas might retain aromatase, and estrogen might be produced in the carcinoma cells by aromatase. This estrogen might bind to estrogen receptors in the carcinoma cells, and growth might be accelerated. There are only few reports on hormone responsiveness of gynecological carcinomas. Clinical trials of some aromatase inhibitors are currently

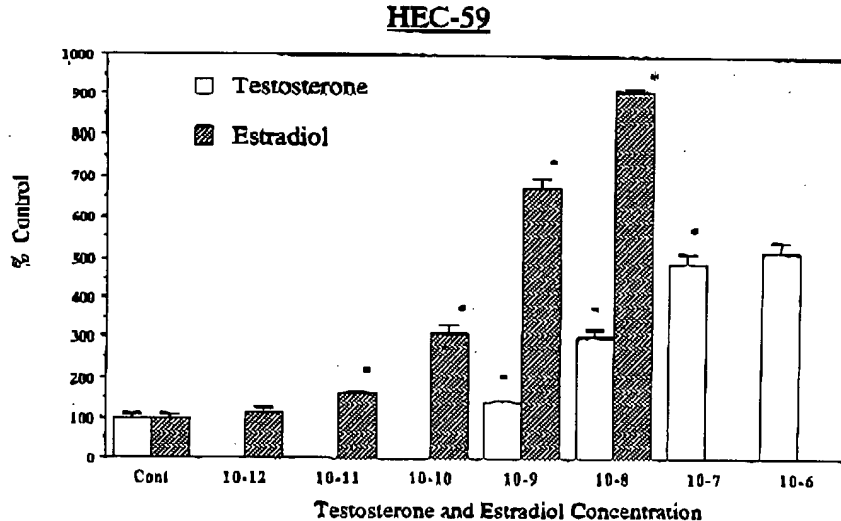


Fig. 1. Response to estradiol and testosterone in DNA synthesis, as measured using the [<sup>3</sup>H]-thymidine incorporation method, in the HEC-59 (Fig. 1) cells. \**p* < 0.01 (Student's *t*-test) comparing to adjacent less concentration of drugs. \*\**p* < 0.05 (Student's *t*-test) comparing to adjacent less concentration of drugs. Each datum was obtained from three independent experiments.

undergoing for breast cancer<sup>17-20</sup>, but not yet for endometrial carcinoma.

In this study, we utilized cultured human carcinoma cell lines derived from uterine endometrium, uterine cervix, ovary, and breast to investigate the levels of aromatase activity and effects of sex steroid hormones on DNA synthesis.

### MATERIALS AND METHODS

#### 1. Cell lines

The human carcinoma cell lines used in this study were breast carcinoma cell line MCF-7 (American Type Culture Collection, Rockville, MD, U.S.A.), endometrial carcinoma cell lines HHUA<sup>21</sup>, Ishikawa<sup>22,23</sup>, OMC-2<sup>24</sup>, and HEC-59<sup>25</sup>, uterine cervical adenocarcinoma cell lines OMC-4<sup>26</sup>, CAC-1, and ovarian carcinoma cell lines 2008<sup>27</sup>, A2780<sup>28</sup>, and HRA<sup>29</sup>. Cells were cultured in Eagle's Minimal Essential Medium (Nissui Co. Ltd. Japan) supplemented with 10% (15% for Ishikawa cell line) fetal bovine serum (FBS), 200 U/ml of penicillin, and 200 µg/ml of streptomycin, and maintained in a humidified 5% CO<sub>2</sub> atmosphere at 37°C.

Steroid free FBS (DCC-FBS) was prepared as follows. 100 ml FBS mixed with 0.25 g activated charcoal (Sigma Chemical Co., St Louis, Mo.) and 0.025 g dextran were stirred at 56°C for 30 min and centrifuged to separate the dextran-coated charcoal pellet

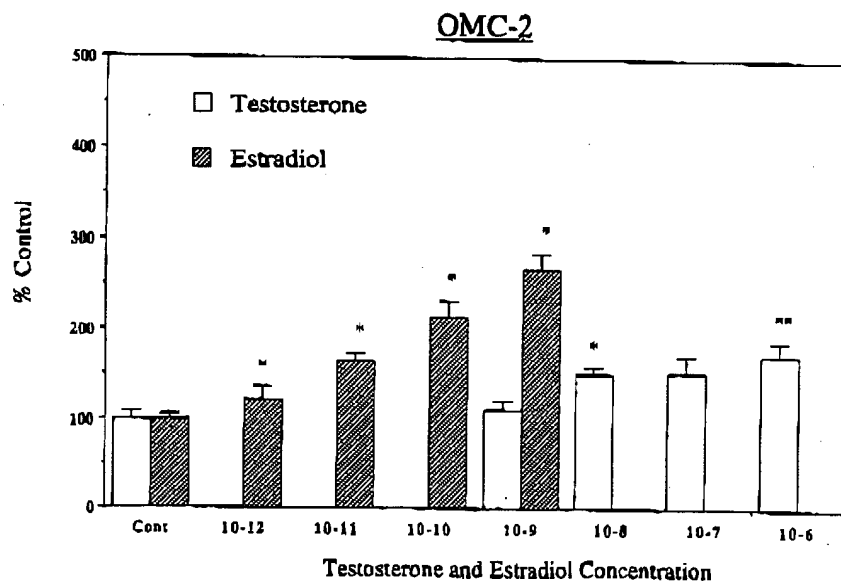


Fig. 2. Response to estradiol and testosterone in DNA synthesis, as measured using the [<sup>3</sup>H]-thymidine incorporation method, in the OMC-2 cells. \**p* < 0.01 (Student's *t*-test) comparing to adjacent less concentration of drugs, \*\**p* < 0.05 (Student's *t*-test) comparing to adjacent less concentration of drugs. Each datum was obtained from three independent experiments.

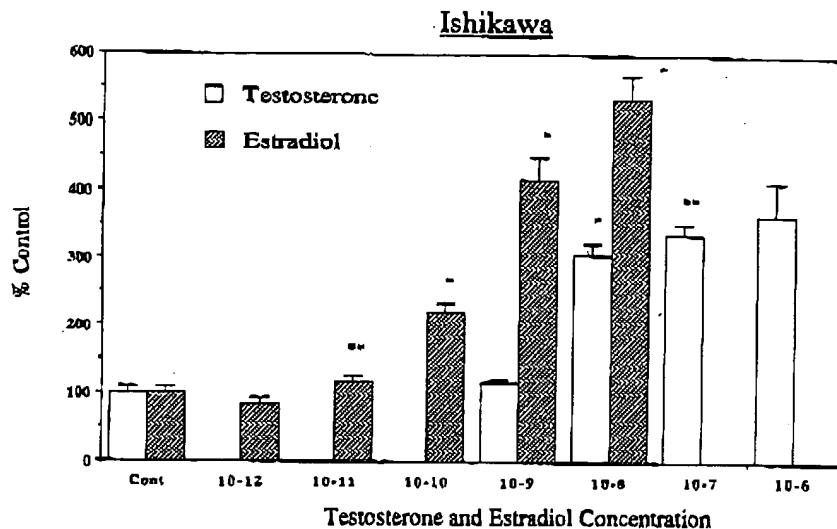


Fig. 3. Response to estradiol and testosterone in DNA synthesis, as measured using the [<sup>3</sup>H]-thymidine incorporation method, in the Ishikawa cells. \*p<0.01 (Student's *t*-test) comparing to adjacent less concentration of drugs, \*\*p<0.05 (Student's *t*-test) comparing to adjacent less concentration of drugs. Each datum was obtained from three independent experiments.

from the supernatant, which was again subjected to the same treatment at 37°C. The charcoal-stripped serum was filtrated through a 0.2  $\mu$ m sterilization unit and stored at -20°C.

## 2. Aromatase assay

For quantitation of aromatase activity, cells were cultured in medium as described above. Twenty four hours prior to aromatase assay, culture medium was changed to the DCC-FBS medium. To start the assay, [<sup>1</sup> $\beta-<sup>3</sup>H] Androstenedione ([<sup>1</sup> $\beta-<sup>3</sup>H] A: 61.05  $\times$  10<sup>6</sup> dpm/nmol, New England Nuclear, Boston, M.A USA) was added to the culture medium at concentration of 100 nM. After 24 hr, 2 ml of culture medium (from a total of 5 ml) were removed and mixed with 1ml of 20% trichloroacetic acid (TCA) and 1ml of 5% charcoal suspension. The mixture was vortexed several times over a 30 min period, centrifuged for 30 min at 3,000 rpm, and the supernatant was removed and filtered. Aliquots were then measured by liquid scintillation counting.$$

## 3. Effect of hormones on DNA synthesis

The effects of estradiol and testosterone on DNA synthesis were investigated using the [<sup>3</sup>H]-thymidine incorporation method. Cells were inoculated into a 96-well dish (Corning Glass Works, Corning, NY, USA) at 10<sup>4</sup> cells/well in a volume of 0.1 ml of medium

December, 1994

## AROMATASE IN GYNECOLOGICAL CANCER CELLS

411

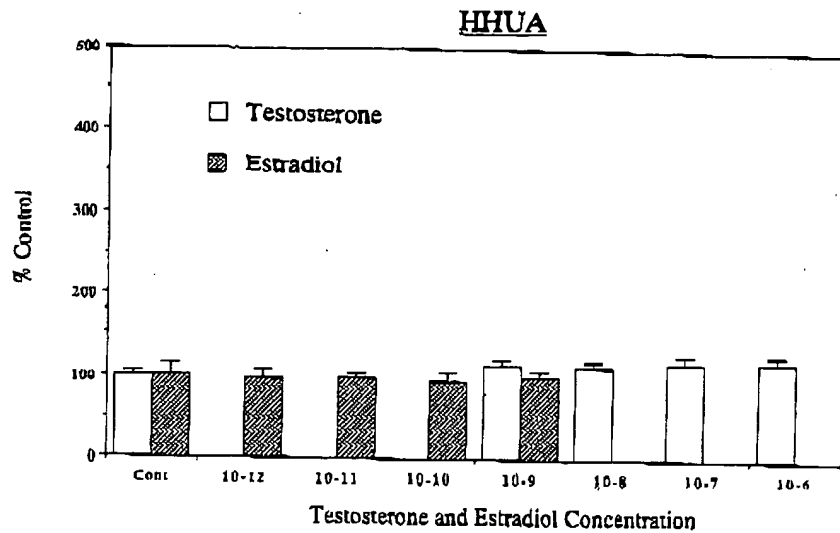


Fig. 4. Response to estradiol and testosterone in DNA synthesis, as measured using the [<sup>3</sup>H]-thymidine incorporation method, in the HHUA cells. \**p* < 0.01 (Student's *t*-test) comparing to adjacent less concentration of drugs, \*\**p* < 0.05 (Student's *t*-test) comparing to adjacent less concentration of drugs. Each datum was obtained from three independent experiments.

Table 1. Estrogen and Testosterone Dependency and Aromatase Activities of Cultured Cell Lines Originated from Gynecological Cancer

Cell line	Origin	Estradiol dependency	Testosterone dependency	Aromatase activity <sup>1</sup>
HEC59	EM Ade. Ca	#	#	195.2 ± 4.5
HHUA	EM Ade. Ca	—	—	69.2 ± 2.4
Ishikawa	EM Ade. Ca	#	#	249.9 ± 10.7
OMC-2	EM Ade. Ca	#	+	514.2 ± 8.2
CAC-1	Ut Cerv. Ade. Ca	—	+	237.4 ± 7.1
OMC-4	Ut Cerv. Ade. Ca	+	—	126.1 ± 3.9
A2780	Ov. Ca	—	—	52.2 ± 1.1
2008	Ov. Ca	—	—	34.0 ± 1.3
HRA	Ov. Ca	#	+	149.8 ± 8.2
MCF-7	Breast Ca	#	#	597.2 ± 10.2

EM Ade. Ca; endometrial adenocarcinoma, Ut Cerv. Ade. Ca; uterine cervical adenocarcinoma, Ov. Ca; ovarian cancer.

<sup>1</sup>; The unit of aromatase activity is fmol/10<sup>7</sup> cells/24 hrs.

# indicates more than 500%, + indicates 200-499%, + indicates 115-199%, — indicates less than 115% enhance when 10<sup>-8</sup> M estradiol or 10<sup>-8</sup> M testosterone was added.

containing DCC-FBS, and cultured for 2 days. Estradiol ( $10^{-12}$  M- $10^{-9}$  M) or testosterone ( $10^{-8}$  M- $10^{-6}$  M) was then added to each well and cultured for an additional 4 days.  $^3$ H-thymidine (0.67  $\mu$ Ci, New England Nuclear) was added to the culture medium for 1 hr, the medium was removed. Wells were washed twice with phosphate-buffered saline, once with ethanol, and twice with 5% TCA. Then the remaining non-acid soluble material was solubilized with 1N sodium hydroxide, and aliquots were quantitated by liquid scintillation counting.

## RESULTS

### 1. Effects of estradiol and testosterone on DNA synthesis

Figures 1-4 show [ $^3$ H] thymidine incorporation of endometrial carcinoma cell lines, HEC-59, Ishikawa, OMC-2, and HHUA cells. Estrogen-dependency, testosterone-dependency, and aromatase activity of each cell line are summarized in Table 1. The cell lines HEC-59, MCF-7, OMC-2, and HRA exhibited a high level of responsiveness to estradiol, with more than a 250% increase in DNA synthesis with the addition of estradiol at  $10^{-9}$  M. The cell lines OMC-4, and Ishikawa exhibited a 115-249% enhancement of DNA synthesis, and the cell lines HHUA, A2780, 2008, and CAC-1 did not show any response to estradiol. Testosterone at  $10^{-6}$  M also enhanced DNA synthesis in the HEC-59 and MCF-7 cell lines by more than 200%, but less than 150% in all of the other cell lines. For all of the cell lines studied, response to testosterone was less than that to estradiol. Testosterone responsiveness correlated well with the level of aromatase activity and estradiol responsiveness, except for the CAC-1 cell lines.

### 2. Aromatase activity

The MCF-7 breast carcinoma cell line demonstrated the highest level of aromatase activity, 597 fmol/ $10^7$  cells/24 hr, among the 10 cell lines studied. Among the gynecologic carcinoma cell lines, OMC-2 demonstrated the highest aromatase activity, 514 fmol/ $10^7$  cells/24 hr, which was 86% of the MCF-7 activity. The cell lines HEC-59, Ishikawa, HRA, CAC-1, and OMC-4 demonstrated activities of 126-321 fmol/ $10^7$  cells/24 hr, 21-54% of the MCF-7 activity. The remaining cell lines, HHUA, A2780, and 2008 demonstrated very low activity, 34-69 fmol/ $10^7$  cells/24 hr, which was only 5.7-11.6% of the MCF-7 activity. There was no correlation between the level of aromatase activity and tissue of origin of the cell lines (Table 1).

## DISCUSSION

The human breast carcinoma cell line MCF-7 is well characterized in regard to its estrogen responsiveness and the ability to synthesize endogenous estrogen with aromatase<sup>20,21</sup>. Although there have been a significant number of reports concerning this phenomenon in MCF-7 cells, data on the ability of gynecological carcinoma cell lines to



carry out aromatase-mediated estrogen biosynthesis are scant. Currently, it is not clear whether aromatase is involved in the growth stimulation of gynecological carcinoma cell lines. In this report, aromatase activity and the effects of estradiol and testosterone on DNA synthesis were verified in gynecological carcinoma cell lines.

Although the HEC-59 and Ishikawa cell lines showed the strongest response to estradiol and testosterone in DNA synthesis, the aromatase activity level was moderate (195.2 and 249.9 fmol/10<sup>7</sup> cells/24 hrs. respectively) and was lower than that of the MCF-7 and OMC-2 cell lines (597.2 and 514.2 fmol/10<sup>7</sup> cells/24 hrs. respectively). On the other hand, response to estradiol in DNA synthesis was moderate in the OMC-2 cell line, aromatase activity was high.

The cell lines with low aromatase activity, namely HHUA, A2780 and 2008 (69.2, 52.2 and 34.0 fmol/10<sup>7</sup> cells/24 hrs. respectively), did not demonstrate estrogen responsiveness. These data indicate that high aromatase activity correlates with a greater response to estradiol and testosterone in DNA synthesis.

Nine cell lines originating from gynecological carcinomas were investigated in this study, and the HEC-59, OMC-2, and HRA cell lines demonstrated high hormone responsiveness. The MCF-7 breast carcinoma cell line is widely used for the investigation of aromatase activity and the effects of aromatase inhibitors on cell growth.

It is difficult to estimate the level of aromatase activity which is required for cell growth, but the HEC-59, OMC-2, and HRA cell lines may possess an adequate amount of aromatase to convert androgen to estrogen for cell growth. Some aromatase inhibitors might therefore be effective in inhibiting the growth of these cell lines.

It is reported that estrogen biosynthesis is inhibited by aromatase inhibitors *in vivo* and *in vitro*<sup>10,31-33</sup>, and anti-tumor effect of aromatase inhibitors has also been reported<sup>10,32,34</sup>. Nowadays, aromatase inhibitors are on clinical use for breast cancer<sup>10,32,34</sup>. The principal site of estrogen biosynthesis in postmenopausal women is adipose tissue<sup>12,35,36</sup>, the most frequent age for gynecological cancer is also postmenopause. Aromatase inhibitors may be useful for gynecological cancer therapy not only by inhibiting aromatase activity of the cancer cells, but also by decreasing the aromatase activity in adipose tissue, resulting in a decreased serum estrogen level.

**Acknowledgements :** We thank Prof. Yoshiteru Terashima for his excellent advice to this study, Miss Kumiko Ishii for technical assistance. And we would also like to thank Dr. Jeff Boyd and Mr. Eric Souw for their English assistance in the preparation of this manuscript. The present study was presented at the 3rd International Conference on Aromatase, Bologna, Italy, June 1992.

#### REFERENCES

- 1) Parazzini F, Vecchia CL, Bocciarelli L, Franceschi S. The epidemiology of endometrial cancer. *Gynecol Oncol* 1991; 41: 1-16.

- 2) Edman CD, McDonald PC. Effect of obesity on conversion of plasma androstenedione to estrone in ovulatory and anovulatory young women. *Am J Obstet Gynecol* 1978; 130: 456-60.
- 3) Judd HL, Davidson BJ, Frumar AN, Shamonki IM, Lagasse LD, Ballon SC. Serum androgens and estrogens in postmenopausal women with and without endometrial cancer. *Am J Obstet Gynecol* 1980; 136: 859-71.
- 4) Deppe G. Chemotherapy of gynecologic cancer. 2nd ed. New York: Wiley-Liss. 1990: 155-74.
- 5) Kleine W, Maier T, Geyer H, Pfeleiderer A. Estrogen and progesterone receptors in endometrial cancer and their prognostic relevance. *Gynecol Oncol* 1990; 38: 59-65.
- 6) Brodie AMH. Aromatase inhibition and its pharmacologic implications. *Biochem Pharmacol* 1985; 34: 3213-9.
- 7) Johnston JO, Metcalf BW. Aromatase: a target enzyme in breast cancer. In: Novel approaches to cancer therapy. New York: Academic Press, 1984: 307-28.
- 8) Yamamoto T. Estrogen biosynthesis in human breast cancer. *J Kyoto Pref Univ Med* 1982; 91: 507-13.
- 9) Yamamoto T, Osawa Y, Yarborough C, Kitawaki J, Urabe M, Okada H, et al. Relation between estrogen biosynthesis and steroid receptors in human breast cancer. *J Kyoto Pref Univ Med* 1989; 98: 763-9.
- 10) McNatty KP, Baird DT, Bolton A, Chambers P, Corker CS, McLean H. Concentrations of oestrogens and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. *J Endocrinol* 1976; 71: 77-85.
- 11) Miller WR. Aromatase activity in breast tissue. *J Steroid Biochem Molec Biol* 1991; 39: 783-90.
- 12) Thijssen JHH, Blankenstein MA, Donker GH, Droszewski J. Endogenous steroid hormones and local aromatase activity in the breast. *J Steroid Biochem Molec Biol* 1991; 39: 799-804.
- 13) Grodin JM, Siiteri PK, McDonald PC. Source of estrogen in postmenopausal women. *J Clin Endocrinol Metab* 1973; 36: 207-14.
- 14) Simpson ER, Merrill JC, Hollub AJ, Graham-Lorence S, Mendelson CR. Regulation of estrogen biosynthesis by human adipose cells. *Endocr Rev* 1989; 10: 136-48.
- 15) Roselli CE, Horton LE, Resco JA. Distribution and regulation of aromatase activity in the rat hypothalamus and limbic systems. *Endocrinology* 1985; 117: 2471-77.
- 16) Yamaki J, Yamamoto T, Okada H. Aromatization of androstenedione by normal and neoplastic endometrium of the uterus. *J Steroid Biochem* 1985; 22: 63-6.
- 17) Brodie AMH, Dowsett M, Coombes C. Aromatase inhibitors as new endocrine therapy for breast cancer. *Cancer Treat Res* 1988; 39: 51-65.
- 18) Santen RJ. Clinical use of aromatase inhibitors: current data and future perspectives. *J Enzyme Inhibition* 1990; 4: 79-99.
- 19) Brodie AMH, Banks PK, Inkster SE, Dowsett M, Coombes C. Aromatase inhibitors and hormone-dependent cancers. *J Steroid Biochem Molec Biol* 1990; 37: 327-33.
- 20) Miller WR. Endocrine treatment for breast cancers: biological rationale and current progress. *J Steroid Biochem Molec Biol* 1990; 37: 467-80.
- 21) Ishiwata I, Ishiwata C, Soma M, Arai J, Ishikawa H. Establishment of human endometrial adenocarcinoma cell line containing estradiol-17B and progesterone receptors. *Gynecol Oncol* 1984; 17: 281-90.
- 22) Holiaka CF, Hata H, Kuramoto H, Gurpide B. Response to estradiol in a human endometrial adenocarcinoma cell line (Ishikawa). *J Steroid Biochem* 1986; 24: 85-9.
- 23) Nishida M, Kasahara K, Kaneko M, Iwasaki H, Hayashi K. Establishment of a new human endometrial adenocarcinoma cell line, Ishikawa cells, containing estrogen and progesterone receptors. *Acta Obstet Gynaecol Jpn* 1985; 37: 1103-11.
- 24) Yamada T, Ueda M, Maeda T, Okamoto Y, Miyawaki Y, Sugimoto O, et al. Establishment and characterization of CA 125 producing cell line (OMC-2) originating from a human endometrial adenocarcinoma. *Asia-Oceania J Obstet Gynaecol* 1989; 15: 403-16.
- 25) Morisawa T. The results of primary culture of endometrial adenocarcinoma and characterization of its established cell line. *J Jpn Soc Clin Cytol* 1987; 26: 433-42.
- 26) Yamada T, Ueda M, Maeda T, Miyawaki Y, Misaki O, Sugimoto O, et al. Establishment and

- characterization of a cell line (OMC-4) originating from a human adenocarcinoma of the uterine cervix. *Acta Obstet Gynecol Jpn* 1987; 39: 859-60.
- 27) Andrews PA, Velury S, Mann SC, Howell SB. Cis-Diamminedichloroplatinum (II) accumulation in sensitive and resistant human ovarian carcinoma cells. *Cancer Res* 1988; 48: 68-73.
  - 28) Louis KG, Hamilton TC, Winker MA, Behrens BC, Tsuruo T, Ozols RF, et al. Adriamycin accumulation and metabolism in adriamycin-sensitive and-resistant human ovarian cancer cell lines. *Biochem Pharmacol* 1986; 35: 467-72.
  - 29) Kikuchi Y, Kizawa I, Oomori K, Miyauchi M, Kita T, Kato K, et al. Establishment of a human ovarian cancer cell line capable of forming ascites in nude mice and effects of tranexamic acid on cell proliferation and ascites formation. *Cancer Res* 1987; 47: 592-6.
  - 30) MacIndoe JH. Estradiol formation from testosterone by continuously cultured human breast cancer cells. *J Clin Endocr Metab* 1979; 49: 272-7.
  - 31) Kitawaki J, Fukuoka M, Yamamoto T, Honjo H, Okada H. Contribution of aromatase to the deoxyribonucleic acid synthesis of MCF-7 human breast cancer cells and its suppression by aromatase inhibitors. *J Steroid Biochem Molec Biol* 1992; 42: 267-77.
  - 32) Dowsett M. Inhibitors of steroidogenic enzymes for the treatment of breast cancer. *J Steroid Biochem Molec Biol* 1991; 39: 805-9.
  - 33) Bhatnagar AS, Haasler A, Schieweck K. Inhibition of aromatase in vitro and in vivo by aromatase inhibitors. *J Enzyme Inhib* 1990; 4: 79-86.
  - 34) Lonning PE, Dowsett M, Powles TJ. Postmenopausal estrogen biosynthesis and metabolism: alterations caused by aromatase inhibitors used for the treatment of breast cancer. *J Steroid Biochem* 1990; 35: 355-66.
  - 35) Judd HL, Lucas W, Yen SSC. Serum 17 $\beta$ -estradiol and estrone levels in postmenopausal women with and without endometrial cancer. *J Clin Endocrinol Metab* 1976; 43: 272-8.
  - 36) Judd HL, Shamonki IM, Frumar AM, Lagasse LD. Origin of serum estradiol in postmenopausal women. *Obstet Gynecol* 1982; 59: 680-6.